antitrypsin. The selection of this membrane prevents the transfer of albumin from the third stream but allows the α-1-antitrypsin to pass through where it is collected in a fourth stream. Following this separation, substantially pure albumin remains in the third stream and substantially pure α-1-antitrypsin is collected in the fourth stream.

Once albumin and α-1-antitrypsin have been separated into their separate streams, third and fourth consecutively, IgG can then be separated from the treated first stream. This is achieved by disconnecting the first apparatus from the second and third apparatus and changing the pH of the buffer. A pH 4.6 GABA/Acetic acid buffer is suitable and the potential is reversed as per the protocol for a normal second phase IgG separation.

All three proteins, albumin, α-1-antitrypsin, and IgG, can be separated to single band purity with over 80% yield using the coupled apparatus. Both albumin and α-1-antitrypsin take about three hours to purify whilst IgG takes several hours longer due to the need to separate the three apparatus once the albumin and α-1-antitrypsin have been separated.

Conclusions

A method to rapidly purify albumin, IgG and α-1-antitrypsin from a single volume of plasma has been established. The minimisation of waste and the removal of various processing steps including ethanol precipitation and ultra-filtration demonstrate the potential of Gradiflow™ technology in the large-scale purification of blood proteins. Optimisation of the process would allow the removal of specific families and even species of the immunoglobulins. Further processing of Gradiflow™ waste fractions may allow the removal of many other important plasma molecules, providing a means by which to maximise the potential of plasma as a biopharmaceutical source. The high specificity of Gladiflow™ technology could allow specific molecules to be targeted and removed by applying suitable strategies.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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What is claimed is:

1. A method of separating components from plasma, the method comprising the steps:

(a) placing the plasma in a first solvent stream, the first solvent stream being separated from a second solvent stream by a first electrophoretic separation membrane having a molecular mass cut-off less than the molecular mass of albumin and a restriction membrane having a molecular mass cut-off less than the first electrophoretic separation membrane;

(b) selecting a buffer for the first solvent stream having a pH greater than the pI of albumin;

(c) applying an electric potential between the two solvent streams causing movement of albumin and α-1-antitrypsin through the first electrophoretic membrane into the second solvent stream while biomolecules having a molecular mass greater than albumin and α-1-antitrypsin are substantially retained in the first solvent stream, or if entering the first electrophoresis membrane, being substantially prevented from passing through the first electrophoresis membrane, wherein biomolecules in the plasma having a molecular mass less than albumin and α-1-antitrypsin are caused to move through the first separation membrane and the restriction membranes to a waste collection;

(d) optionally, periodically stopping and reversing the electric potential to cause movement of biomolecules having a molecular mass greater than albumin and α-1-antitrypsin having entered the first electrophoresis membrane to move back into the first solvent stream, wherein substantially not causing any albumin or α-1-antitrypsin that have entered the second solvent stream to re-enter first solvent stream;

(e) maintaining steps (c) and optionally (d) until the desired amount of albumin and α-1-antitrypsin have been collected as an albumin/α-1-antitrypsin pool and biomolecules having a molecular mass less than albumin and α-1-antitrypsin have been removed from the first solvent stream to form a treated plasma;

(f) placing the treated plasma in a third solvent stream, the third solvent stream being separated from a fourth solvent stream by a second electrophoretic separation membrane having a molecular mass cut-off less than the molecular mass of immunoglobulins;

(g) selecting a buffer for the third solvent stream having a pH above neutral;

(h) applying an electric potential between the third and fourth solvent streams causing movement of biomolecules having a molecular mass less that that of immunoglobulins in the treated plasma through the second electrophoretic separation membrane into the fourth solvent stream while immunoglobulins and other biomolecules having a molecular mass greater than immu-

noglobulins are substantially retained in the third solvent stream, or if entering the second electrophoresis separation membrane, being substantially prevented from passing through the second electrophoresis separation membrane;

(i) optionally, periodically stopping and reversing the electric potential to cause movement of immunoglobulins and other biomolecules having a molecular mass greater than immunoglobulins having entered the second electrophoresis separation membrane to move back into the third solvent stream, wherein substantially not causing any biomolecules having a molecular mass less than immunoglobulins that have entered the fourth solvent stream to re-enter third solvent stream;

(j) maintaining steps (h) and optional (i) until the desired amount of biomolecules having a molecular mass less than immunoglobulins have been removed from the third upstream to form an immunoglobulins concentrate;

(k) removing the biomolecules from the fourth solvent stream;

(l) replacing the second electrophoretic separation membrane with a third electrophoretic separation membrane having a molecular mass cut-off greater than the molecular mass of immunoglobulins;

(m) selecting a buffer for the immunoglobulins concentrate having a pH below neutral;

(n) applying an electric potential between the immunoglobulins concentrate in the third solvent stream and a fresh fourth solvent stream causing movement of immunoglobulins in the immunoglobulins concentrate in the third solvent stream through the third electrophoretic separation membrane into the fresh fourth solvent stream while biomolecules having a molecular mass greater than immunoglobulins are substantially retained in the third solvent stream, or if entering the third electrophoresis separation membrane, being substantially prevented from passing through the third electrophoresis separation membrane;

(o) optionally, periodically stopping and reversing the electric potential to cause movement of biomolecules having a molecular mass greater than immunoglobulins having entered the third electrophoresis membrane to move back into the treated third solvent stream, wherein substantially not causing any immunoglobulins that has entered the fresh fourth solvent stream to re-enter treated third solvent stream;

(p) maintaining steps (n) and optional (o) until the desired amount of immunoglobulins have been moved to the fresh fourth downstream;

(q) placing the albumin/α-1-antitrypsin concentrate in a fifth solvent stream, the fifth solvent stream being separated from a sixth solvent stream by a fourth electrophoretic separation membrane having a molecular mass cut-off less than the molecular mass of albumin;

(r) selecting a buffer for the fifth solvent stream having a pH greater than neutral;

(s) applying an electric potential between the fifth and sixth solvent streams causing movement of α-1-antitrypsin through the fourth electrophoresis separation membrane into the sixth solvent stream while albumin is substantially retained in the fifth solvent stream, or if entering the fourth electrophoresis separation membrane, being substantially prevented from passing through the fourth electrophoresis separation membrane;

(t) optionally, periodically stopping and reversing the electric potential to cause movement of albumin having entered the fourth electrophoresis separation membrane to move back into the fifth solvent stream, wherein substantially not causing any α-1-antitrypsin that has entered the sixth solvent stream to re-enter the fifth solvent stream; and

(u) maintaining steps (s) and optionally (t) until the desired amount of albumin remains in the fifth solvent stream and the desired amount of α-1-antitrypsin has have been removed to the sixth solvent stream.

**2**. The method according to claim **1** wherein steps (q) to (u) are carried out after steps (a) to (e).

**3**. The method according to claim **1** wherein the plasma is a pooled human plasma sample.

**4**. The method according to claim **1** wherein the first electrophoresis separation membrane of step (a) has molecular mass cut-off of about 75 kDa and the restriction membrane has a molecular mass cut off of about 50 kDa.

**5**. The method according to claim **1** wherein the buffer in step (b) has a pH of 9.

**6**. The method according to claim **5** wherein the buffer is a Tris-borate buffer.

**7**. The method according to claim **1** wherein the second electrophoresis separation membrane of step (f) has a molecular mass cut-off of 200 kDa.

**8**. The method according to claim **1** wherein the third electrophoresis separation membrane of step (l) has a molecular mass cut-off of 500 kDa.

**9**. The method according to claim **1** wherein the buffer of the third solvent stream in step (g) has a pH of 9.

**10**. The method according to claim **1** wherein the buffer of the immunoglobulins concentrate of step (m) has a pH of less than 5.

**11**. The method according to claim **10** wherein buffer has a of pH 4.6.

**12**. The method according to claim **1** wherein the fourth electrophoresis separation membrane of step (q) has molecular mass cut-off of about 50 kDa.

**13**. The method according to claim **1** wherein the buffer of the fifth solvent stream in step (r) has a pH of 8.0.

**14**. The method according to claim **13** wherein the buffer is a Tris-borate buffer.

**15**. The method according to claim **1** wherein a potential of 250 volts is applied in steps (c), (h), (n) and (s).

**16**. The method according to claim **1** wherein the immunoglobulins are immunoglobulin G (IgG).

**17**. The method according to claim **1** wherein yields of albumin, immunoglobulins and α-1-antitrypsin from plasma are at least 70% and purity of at least 90%.

**18**. The method according to claim **1** wherein albumin, immunoglobulins and α-1-antitrypsin are separated from plasma in less than 1 day.

**19**. The method according to claim **18** wherein albumin, immunoglobulins and α-1-antitrypsin are separated from plasma in less than 12 hours.

**20**. The method according to claim **18** wherein albumin, immunoglobulins and α-1-antitrypsin are separated from plasma in less than 6 hours.

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